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Kinetics of radioiodinated heptadecanoic acid and metabolites in the normal and ischaemic canine heart

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This study was undertaken to elucidate if the myocardial elimination rate of the radioactivity after administration of radioiodinated heptadecanoic acid was related to beta-oxidation of the fatty acid or related to washout of free radioiodide. In samples of normal and ischaemic myocardium the distribution of the radioactivity over free radioiodide, heptadecanoic acid and lipids was determined. In normal myocardium the major component was free radioiodide, only a small percentage being heptadecanoic acid. In ischaemic myocardium more radiolabelled lipids were present and less free iodide when compared with normal myocardium. The percentage heptadecanoic acid was slightly increased.

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Introduction

Oxidation of long-chain free fatty acids (FFAs) contributes under normal physiological conditions for about 80% to the production of energy-rich phosphate bonds. In contrast, in ischaemia uptake and degradation of FFA is diminished. Hence, FFAs labelled with a radioisotope, can be used for cardiac imaging and for *in vivo* investigation of myocardial lipid metabolism. One of these fatty acids is heptadecanoic acid labelled with radioiodine in the omega position (I-HDA). With this agent, scintigraphic images can be obtained, clearly distinguishing between normal and ischaemic or infarcted myocardium[1–3].

The elimination of the radioactivity from the myocardium is used as indicator of myocardial metabolism. A few arguments favour this concept: in ischaemic myocardium the rate of elimination is delayed when compared with normal myocardium[4]. Simultaneous administration with glucose-insuline also decreases the rate of elimination[5], demonstrating the effect of substrate availability in myocardial metabolism. Other data, however, suggest that the elimination rate is not closely related to fatty acid metabolism but merely related to washout of the radiolabel, being split off during beta-oxidation[6] of the carbon chain.

The aim of this study was to establish the relation between the elimination rate and I-HDA metabolism. We did so by analysing in normal and ischaemic myocardium the content of I-HDA, its metabolite free radioiodide and the various radiolabelled lipids.

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Material and methods

PREPARATION OF I-131-HEPTADECANOIC ACID

I-HDA was prepared by non-isotopic halogen exchange using 17-bromo-HDA as the parent compound. Free iodide was removed by eluation over Sephadex A25. I-HDA was dissolved in a minimal volume of ethanol and physiological saline and added to 20% HSA. The final solution contained less than 5% ethanol.

ANIMAL EXPERIMENTS

Twelve male mongrel dogs were studied after overnight fasting (weight between 21 and 35 kg). Dogs were anaesthetized with 30 mg kg^{-1} sodium pentobarbital *i.v.* after premedication with atropine (0·5 mg), droperidol (2·5 mg) and fentanyl (0·05 mg).

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Anaesthesia was maintained with nitrous oxide and oxygen in combination with trifluoroethane 2%. Pulmonary ventilation was maintained with Pulmonat positive pressure respirator. A catheter was placed in a carotid artery for arterial pressure measurements and blood sampling.

Electrocardiograms were monitored continuously. Three groups of dogs were studied:

group 1—6 dogs without a coronary occlusion;
group 2—3 dogs with a coronary occlusion for a period of 5 min;
group 3—3 dogs with a coronary occlusion for a period of 6 h.

The chest was opened by an incision through the fifth left intercostal space, the pericardium opened and fixed to the chest wall to form a pericardial craddle. Dogs of groups 2 and 3 underwent occlusion of the anterior descending branch (LAD) of the left coronary artery, just distal to the first diagonal branch.

In all dogs, 3 to 9 mCi I-HDA was rapidly injected into the left atrium: in dogs of group 1 this was after exposing the heart, in dogs of groups 2 and 3 this was after the coronary occlusion period.

In dogs of group 1 serial biopsies were taken with a fast spinning, hollow needle (bore 2·11 mm) from myocardium perfused by the LAD (weight between 15 and 50 mg). These biopsies were rapidly stored in liquid nitrogen. Samples were taken at approximately 1, 2, 5, 10, 20 and 30 min after I-HDA injection. Dogs of groups 2 and 3: 5 min after injection of I-HDA 30 ml iced KCl solution was injected into the left ventricle. Simultaneously liquid nitrogen was poured over the heart, which was then rapidly excised. The heart was immersed in iced water and washed to remove blood. From injection of KCl to immersion in water took less than 40 s.

From the ischaemic myocardium, perfused by the LAD, three pieces of myocardium were excised, measuring approximately 1 × 1 cm. From an area perfused by the left circumflex artery three pieces of the same size were taken.

All samples of the dogs were weighed and counted in a gamma well counter (LKB Ultrogamma 1280), using a window setting suitable for the I-131 isotope. Count time for biopsies of group 1 dogs was 2 min, for those of group 2 and 3 1 min.

BLOODSAMPLING

In dogs of group I 2 ml bloodsamples were drawn from the arterial catheter, at the same time the biopsies were taken.

CHEMICAL ANALYSIS

Myocardial biopsies

After freezing, myocardial biopsies were ground in a mortar. During pulverization 2 ml of chloroform/methanol 2/1 v.v. was added. After addition of 0·02 N HCl, 1 ml 48% urea and after being centrifuged, the aqueous fraction containing the free radioiodide, the pellet and the organic fraction containing I-HDA and lipids were separated. The latter fraction was dried by evaporation under a stream of nitrogen until a minimal volume was left.

The solution containing the lipid fraction was brought onto a silicagel chromatography plate (Merck 60 F 254) by use of the eluent hexane/ether/acetic acid 80/20/1 v.v. On the chromatography plate I-HDA was identified by comparison with a standard under fluorescence. Approximate Rf values: phospholipids 0·0, I-HDA, 0·50, (mono, di, tri)-glycerides between 0·60 and 0·90 and cholesterol esters 0·95. I-HDA was isolated by cutting the chromatography plate in appropriate strips. The strips of the fatty acid esters were combined and will be further referred to as 'lipids'. Count rates of the aqueous fraction—containing the free radioiodide—, of I-HDA and of the radiolabelled lipids were obtained in the gamma well counter. Count time was 3 min per sample. Corrections were made for physical decay.

Bloodsamples

The plasma was separated from the haematocrit by centrifugation. Two hundred microliter plasma was eluated over Sephadex A25, which retained the free radioiodide. The eluent contained the I-HDA. The Sephadex column and the eluent were counted in the gamma well counter. Count time was 3 min, and was corrected for decay of the radioisotope.

DATA ANALYSIS

Myocardial biopsies

Count rates of a biopsy obtained prior to chemical analysis were expressed as cpm mg^{-1}. After chemical analysis the count rates of the separate fractions of a biopsy were added. The fractions were expressed as a percentage of the cpm mg^{-1} of the biopsy obtained prior to pulverization.

Bloodsamples

Total plasma activity was expressed as a percentage of the plasma sample with the highest counts in

Table 1 Dogs of group 1: count rates in myocardial biopsies as a percentage of the maximal count rate

	Time (min)					
	1	2	5	10	20	30
Total						
mean	71	82	97	85	70	71
SD	6	8	4	12	16	19
I-HDA						
mean	9	6	4	2	1	1
SD	8	2	1	1	1	1
Free iodide						
mean	46	55	65	51	35	29
SD	13	19	17	15	11	6
I-lipids						
mean	16	21	28	32	34	40
SD	2	5	6	7	7	8

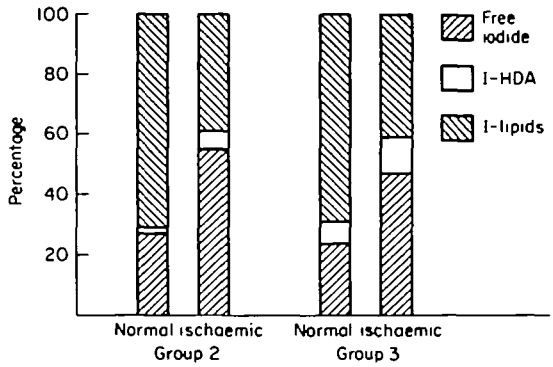

Figure 1 Distribution of radioactivity in normal and ischaemic myocardium in dogs after a 5 min and 6 h occlusion period of the LAD. The proportion of free radioiodide (I^-), I-HDA and radioiodinated lipids are given.

a dog. The aqueous and organic fractions were expressed as a percentage of total plasma activity.

Results

Arterial blood pressure in dogs decreased from 85/62 to 74/60 mmHg during the study.

Table 1 gives the time course of the radioactivity, and the distribution over I-HDA, free iodide, and the radioiodinated lipids in dogs of group 1. Total count rates as obtained prior to chemical work-up increased up to the 5th min, and thereafter were seen to decrease. The half-time value calculated from the 5th to the 30th min was 37 min. I-HDA rapidly decreased from the onset of the study. Free radioiodide was the largest part of the radioactivity and the course paralleled the curve of the total radioactivity. Lipids increased during the study.

Table 2 and Fig. 1 show the count rates of normal and ischaemic myocardium 5 min after injection of I-HDA in dogs of groups 2 and 3.

In normal myocardium the distribution of the radioactivity was the same as in dogs of group 1 at 5 min: the major part of the radioactivity originated from free iodide and lipids, a small percentage being I-HDA. In ischaemic myocardium the percentage I-HDA was slightly higher, free iodide, predominantly present in normal myocardium, was less and lipids contributed most to the radioactivity. No statistical significant difference was observed between dogs with a 5 min (group 2) and dogs with a 6 h occlusion period (group 3). Figure 2 shows the course of

Table 2 Dogs of groups 2 and 3: count rates in normal and ischaemic myocardium 5 min after I-HDA administration

	Group 2		Group 3	
	normal	ischaemic	normal	ischaemic
I-HDA				
mean	2	6	7	12
SD	1	5	3	6
Free iodide				
mean	71	39	69	41
SD	22	6	5	9
I-lipids				
mean	27	55	24	47
SD	22	18	3	10

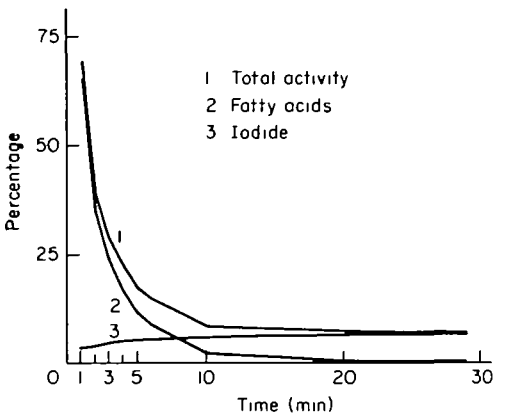

Figure 2 Time course of the radioactivity in blood in normal dogs during 30 min. Data are expressed as a percentage of the counts of the first blood sample. Separation of the radioactivity in I-HDA and I^- is given.

radioactivity in plasma in group 1 dogs. In the first 10 min a rapid decrease was seen followed by a plateau phase. In the first minutes the major component was I-HDA: mean half-life of I-HDA of group 1 dogs was 2·4 min. After 20 min almost 100% of the radioactivity was radioiodide.

Discussion

Radiolabelled FFAs are used to explore non-invasively myocardial fatty acid metabolism in men. C-11-palmitic acid is the standard used to quantify *in vivo* fatty acid metabolism in normal and pathologically altered myocardium; however, the high costs of production and detection facilities limit the general use of these tracers. One of the commonly used alternatives is heptadecanoic acid labelled with the gamma emitter radioiodine in the omega position. As the iodide molecule structurally resembles a methyl group, minimal stearic hindrance is present, which theoretically gives a behaviour identical to the natural fatty acid analogue.

The elimination of the radioactivity is supposed to reflect cardiac oxidative metabolism of the fatty acids because radioiodide is split off during beta-oxidation[7]. After being split off, iodide leaves the cell and enters the circulation. This oxidative concept is supported by the fact that glucose infusion and ischaemic conditions delay the rate of elimination.

This study was undertaken to ascertain whether the elimination rate was related to oxidative metabolism or to washout of free radioiodide.

NORMAL DOGS

The increase in total radioactivity of the biopsies (Table 1) was due to uptake of recirculating I-HDA in arterial blood (Fig. 2). In the serial myocardial biopsies I-HDA rapidly decreased to low levels, whereas lipids slightly increased during the study. The major part of the radioactivity consisted of free radioiodide which paralleled the course of the total radioactivity (Table 1). The data indicate that after entering the cell I-HDA is either stored in lipids or oxidized, releasing free radioiodide, thus showing a behaviour similar to the 'natural' fatty acids. The results suggest that oxidation rate is a very fast process and—as the lipids remained constant from the 5th until the 30th min—that the washout of radioiodide from the myocardial cell into the circulation determines the elimination rate as observed during a scintigraphic study.

ISCHAEMIC DOG MYOCARDIUM

In an ischaemic myocardium, FFA metabolism is markedly altered. Uptake and metabolic breakdown of FFAs is decreased and a larger fraction of FFAs is stored in various lipids[8]. The same pattern was seen in our experiments (Fig. 1 and Table 2): compared with normal myocardium a larger part of the radioactivity was present in the lipids. The contribution of free iodide was seen to decreased, which indicated that the oxidation of I-HDA was diminished.

Although I-HDA was administered in group 2 dogs after 5 min and in group 3 dogs after 6 h no difference in metabolic pattern was observed between the two groups in the ischaemic myocardium. For the group 3 dogs, one may speculate that uptake and metabolism of I-HDA occurred only in the ischaemic cells and not in the necrotic cells. This would give the same results as for group 2 dogs.

In summary: the metabolic behaviour of I-HDA with regard to uptake and distribution in normal and ischaemic myocardium resembles that of the naturally occurring fatty acids. Furthermore, the elimination rate of the radioactivity as observed during a scintigraphic study cannot be related to the rate of beta-oxidation of I-HDA. In contrast, washout of free radioiodide from the myocardial cell into the circulation determines the elimination rate during the studied time period. Alterations in the elimination rate must therefore be interpreted as changes in the iodide concentration gradient and altered membrane function as barriers for iodide washout. Alterations in intracellular iodide con-

centration can be caused by decreased I-HDA uptake and oxidation, or increased storage in the lipids (e.g. under influence of ischaemia and glucose administration). Membrane properties can change during ischaemia and infarction or during pharmacological interventions (halothane anaesthesia).

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